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ORIGINAL ARTICLE

Identification of Differentially Expressed Genes and Biological Pathways in Sanguinarine-treated Ovarian Cancer by Integrated Bioinformatics Analysis

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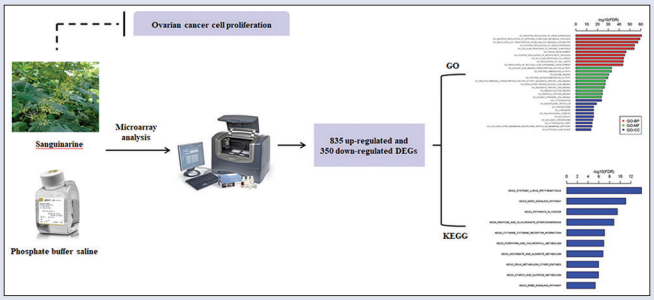
ABSTRACT

Aim: This study was intended to identify potentially target genes and underlying biological pathway of sanguinarine in ovarian cancer. **Methods:** We obtained the expression changes of downstream target genes and underlying biological pathways regulated by control and sanguinarine groups via Affymetrix gene expression profile chip in ovarian cancer cells. An Affymetrix Genechip Agilent mRNA Array was used to recognize differentially expressed genes (DEGs). Afterward, gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were performed for the DEGs using the DAVID database. **Results:** A total of 1185 DEGs between sanguinarine and control groups were identified, including 835 upregulated and 350 downregulated DEGs. The result of GO analysis recommended that the DEGs were mostly enriched in biological processes, including negative regulation of gene expression, nitrogen compound metabolic process, and transcription from RNA polymerase II promoter. Alterations in cellular components (CC) were suggestively enriched in the cytoskeleton and endoplasmic reticulum. The changes in molecular function were suggestively enriched in nucleic acid-binding transcription factor activity, protein dimerization activity, and enzyme binding. The results of the KEGG pathway analysis indicated that the DEGs were mostly concentrated in "Systemic lupus erythematosus," "MAPK signalling pathway," "Pathways in cancer," and "Metabolic pathways." **Conclusion:** The present study provided insights into the mechanism underlying sanguinarine target genes in ovarian cancer cells, which might be used as effective targets for OC diagnosis and treatment.

Key words: Bioinformatics analysis, differentially expressed genes, enrichment analysis, ovarian cancer, sanguinarine

SUMMARY

- In this study, we reported the following achievements:
- A total of 1185 differentially expressed genes (DEGs) between sanguinarine and control groups were identified, including 835 upregulated and 350 downregulated DEGs
- The result of gene ontology analysis recommended that the DEGs were mostly enriched in biological processes
- The results of the Kyoto Encyclopedia of Genes and Genomes pathway analysis indicated that the DEGs were mostly concentrated in "Systemic lupus erythematosus," "MAPK signalling pathway," "Pathways in cancer," and "Metabolic pathways"
- Sanguinarine may serve as a potential therapeutic reagent for epithelial ovarian cancer.



Abbreviations used: OC: Ovarian cancer; DEGs: Differentially expressed genes; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; DAVID database: Database for Annotation, Visualization, and Integrated Discovery; BP: Biological processes; CC: Cell component; MF: Molecular function; ROS: Reactive oxygen species; ER: Endoplasmic reticulum; MAPK: Mitogen-activated protein kinase; JNK: C-Jun N-terminal kinase; STRAP: Serine-threonine kinase receptor-associated protein; MELK; maternal embryonic leucine zipper kinase; FDR: False discovery rate; PCA: Principal component analysis; ERK: Extracellular signal-regulated kinase; p38MAPK: P38 mitogen-activated protein kinase.

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INTRODUCTION

Ovarian cancer is the second common gynecological malignancy but the leading cause of death for all gynecological cancer. Globally, there are approximately 240,000 women diagnosed with ovarian cancer each year, with a 5-year survival rate below 45% and a 15,000 yearly death toll, making it the 8th cause of cancer-associated death in women.[1] However, the cause and underlying molecular events of ovarian cancer are not clear. The high mortality rate of ovarian carcinoma is due to diagnosis at an advanced stage, recurrence, and metastasis, with 5-year rate below 43%.[2-5] Although cytoreductive surgery and platinum- and taxane-based chemotherapies remain the standards of treatment for patients with ovarian cancer, the effectiveness of the aforementioned chemotherapeutic agents is limited by drug resistance.[6] Therefore, developing more effective chemotherapeutic agents for ovarian cancer is instantly desirable.

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Sanguinarine, a member of quaternary benzophenanthridine alkaloid, exhibits anticancer potential through its ability of inducing tumor cells apoptosis, antiproliferative, antiangiogenic, and anti-invasive properties.[7] In lung cancer, sanguinarine is found to play antitumor via inducing the generation of reactive oxygen species (ROS) and the stress of endoplasmic reticulum (ER).[8] It is also stated that sanguinarine can conquer the expression of miR-96-5p and miR-29c-3p and activate the MAPK/JNK signaling pathway to inhibit the proliferation of gastric cancer cells;[9] it also conveyed that sanguinarine can suppress the growth of colorectal cancer cells through disassociation between STRAP and MELK,[10] and sanguinarine also plays the same inhibit effect on the breast cancer via inducing HO-1 expression.[11] In addition, it was found that sanguinarine may inhibit ovarian cancer growth,[12] but the underlying mechanisms are not completely clear.

In recent years, gene chip, a high-throughput, ultramicro technology, has been used to study the molecular mechanisms of drugs by detecting gene regulation and expression networks at the organism level.[13] Bioinformatics tools can be used to link genetic and genomic data for a better understanding of evolutionary facets of molecular and cancer biology. For example, image and signal processing bioinformatics techniques make it possible to extract useful information from massive raw data collection. This tool can also establish and query biological data from the text mining of literature and expression databases to denote gene ontologies. These discoveries have shown great significance in revealing the molecular mechanisms and the effects of drugs on different biological pathways.

To discover the molecular mechanism of sanguinarine-treated ovarian cancer by identifying potentially target genes and underlying biological pathway, we analyzed Affymetrix gene expression profile chip data to get differentially expressed genes (DEGs) between control and sanguinarine groups in ovarian cancer cells. Then, gene ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were performed to describe the molecular mechanisms of sanguinarine-treated ovarian cancer. A total of 1185 DEGs were identified, which may be effective targets for clinical diagnosis and therapy of ovarian cancer.

METHODS

Reagents

Ovarian cancer cell line SKOV3 was purchased from Kunming Institute of Zoology, Chinese Academy of Sciences (Kunming, China). The cells were cultured in Dulbecco's Modified Eagle's Medium (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY, USA) and penicillin–streptomycin (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) in an incubator with 5% CO_2 at 37°C. Sanguinarine (Lot number: 20150607, purity >98%, molecular formula: $C_{20}H_{14}NO_4$) was purchased from RongHe Pharmaceutical Technology (Shanghai, China).

Cell proliferation assays

The cells were divided into two groups: control and sanguinarine groups. Four hours after seeding, the cell groups were treated with phosphate buffer saline or sanguinarine (2.0 μmol/L), respectively. Proliferation of ovarian cancer cells was determined by measuring the absorbance of CCK-8 according to the manufacturer's instructions (Thermo, USA) at 24, 48, 72, 96, and 120 h. A microplate spectrophotometer (BioTek, Winooski, VT, USA) was used to measure the absorbance of each sample at 450 nm, then calculated for survival curves. The experiment was performed in triplicate. Doses of sanguinarine were chosen based on pilot experiments as follows. SKOV3 cells were treated with sanguinarine at doses of 0, 1, 2, 4, 8, 16, 32, and 64 μmol/L. The 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2, 4-disulfonic benzene)-2 h-tetrazolium monosodium salt) (CCK-8) assays were performed to determine cellular proliferation. Pilot experiments showed that the IC_{50} value of sanguinarine was 2 μmol/L. Therefore, this value was chosen for further determination [Figure 1a].

Microarray analysis

Total RNA from the control and sanguinarine group was isolated using TRIzol reagent (Invitrogen, CA, USA) according to the manufacturer's instructions. RNA purity was examined using an Agilent Bioanalyzer 2100 (Agilent Technologies, Inc., Santa Clara, CA, USA). These samples were amplified, labeled, and purified to get biotin labeled amplified RNA using a GeneChip 3'IVT Express Kit (Affymetrix; Thermo Fisher Scientific Inc.). Array hybridization and washes were performed using GeneChip hybridization, wash, and stain kit (Affymetrix; Thermo Fisher Scientific Inc.). Then, the gene chips were scanned with GeneChip Scanner 3000 (Affymetrix; Thermo Fisher Scientific Inc.). The Affymetrix Genechip Agilent mRNA Array was acquired from GeneChem (Shanghai, China). Normalization, background correction, and data cleaning were used to adjust sample signals and guarantee integrity. Furthermore, the gene expression profile is processed by

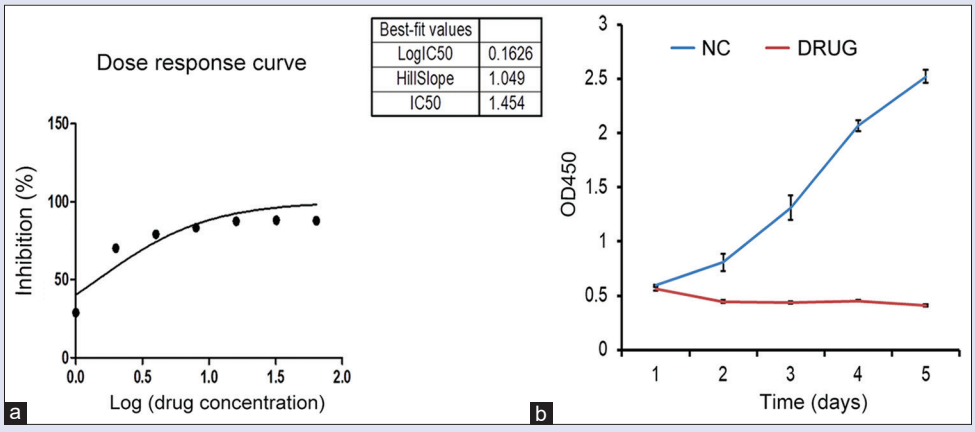

Figure 1: (a) Effect of the drug concentration on the SKOV3 inhibitive rate. The SKOV3 cell line was incubated with different concentrations of the drugs and the inhibitive rate of cell line was tested with the CCK8 assay. (b) Effects of sanguinarine on SKOV3 proliferation. CCK8 assays were performed to determine the cell proliferation abilities in control groups (NC) and sanguinarine groups (DRUG). OD: Optical density

volcano plot, principal component analysis (PCA), and cluster analysis to improve the consistency and accuracy of the studies. Benjamini and Hochberg's false discovery rate (FDR) and fold change were used to filter DEGs. The absolute value of FC (fold change) >3.00 and FDR <0.05 was measured statistically significant.

Gene ontology and Kyoto Encyclopedia of Genes and Genomes pathway analysis

GO categories include three domains: biological processes (BP), cellular components (CC), and molecular functions (MF). Such analyses help us to link and classify DEGs to better understand their biological functions.[14] The Kyoto KEGG pathway analysis is a knowledge base, which is used to study the functional interpretation of genes and genomes as a whole network.[15] Database for Annotation, Visualization, and Integrated Discovery (DAVID; https://david.ncifcrf.gov/), online database, was used to explore the role of sanguinarine-related signaling pathways in OC treatment, with $P < 0.05$ cutoff for significance.

Statistical analysis

All statistical analyses were performed using SPSS (version 18.0; SPSS Inc., Chicago, USA). The significance of the differences was determined using Student's *t*-test (two-sided). GO analysis and KEGG enrichment analysis were analyzed using Fisher's exact test. Data are accessible as means ± standard errors of the meaning. $P < 0.05$ was considered statistically significant.

RESULTS

Sanguinarine suppressed the proliferation of SKOV3 ovarian cancer cells

To confirm that sanguinarine *in vitro* effects on the growth of ovarian cancer cells, we performed CCK8 assays. The assays showed that sanguinarine suggestively suppressed the proliferation of SKOV3 cells in comparison with the control cells [$P < 0.05$, Figure 1b].

Identification of differentially expressed genes in sanguinarine-treated ovarian cancer cells

A total of 1185 DEGs were obtained from Affymetrix GeneChip Agilent mRNA Array, including 835 upregulated and 350 downregulated DEGs. Top ten upregulated genes and top ten downregulated genes are presented in Table S1. We selected expressed gene probes satisfying the screening criterion of Fold Change >3.0 and FDR <0.05 for hierarchical clustering analysis. It was clear that the grouping was rational and data could be directly applied to further analysis [Figure 2a]. The volcano plot displayed that the expressions of DEGs between sanguinarine and controls were significantly different [Figure 2b]. PCA provided a clear

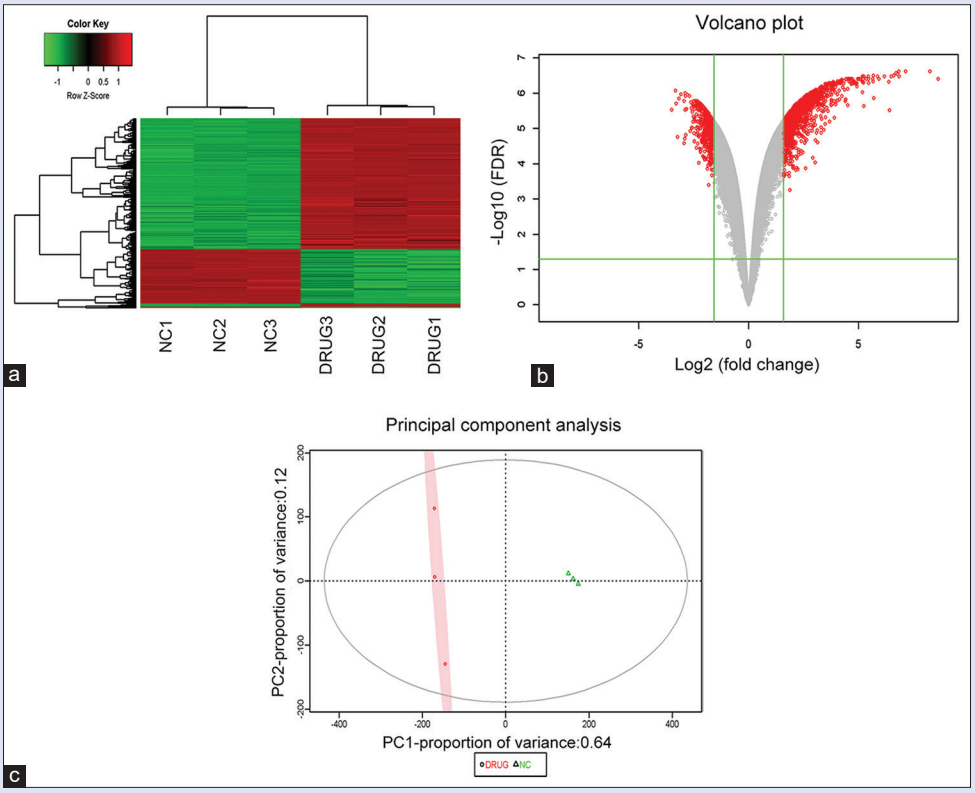

Figure 2: (a) Hierarchical clustering analysis of differentially expressed genes. The hierarchical cluster image shows the differential gene expression profiles in DRUG and NC groups. The heat maps display a color scale: red indicates upregulation, whereas green represents downregulation. The columns and rows in the heat maps represent samples and differentially expressed genes, respectively. Color brightness represents the degree of difference, as shown in the color bar. (b) Difference significance analysis of differentially expressed genes. The volcano plot shows the distribution of differentially expressed genes in grouping comparison and fold change on x-axis. The *P* value on y-axis represents the significance of the difference. Green lines represent the threshold value of *P* and multiple screening, respectively. Each point in the diagram is a detected gene probe. Red dots represent the difference probes above each group. |Fold change|>3 and false discovery rate <0.05. The significance of gene differential expression between experimental and control groups is indicated. (c) Principal component analysis of differentially expressed genes. The red dots represent the DRUG samples and the green dots represent the NC sample. The different experimental groups are separated from one another, the experimental data show good repeatability, thereby showing good specificity

differentiation between sanguinarine and control groups and presented good specificity [Figure 2c].

Gene ontology functional enrichment analysis of differentially expressed genes

GO term analysis[16] is a generally used approach for studying large-scale genomic or transcriptomic data in function that contains three terms: biological process, cellular component, and MF categories. In this study, the changes of DEGs in BP were rich in negative regulation of gene expression, nitrogen compound metabolic process, and transcription from RNA polymerase II promoter. Alterations in cell component (CC) were significantly augmented in the cytoskeleton and ER. The changes in MF were significantly enriched in nucleic acid-binding transcription factor activity, protein dimerization activity, and enzyme binding [Tables S2-S4 and Figure 3].

Further identification and validation of differentially expressed genes using the Kyoto Encyclopedia of Genes and Genomes approach

KEGG signal pathway analysis was performed to analyze the identified DEGs for involved signal pathways. As a result, in the 1185 DEGs, we found that these genes are involved in signal pathways including systemic lupus eryth, MAPK signaling pathway, pathways in cancer, pentose and glucuronate interconversions, cytokine–cytokine receptor interaction, and porphyrin and chlorophyll metabolism, etc., [Table S5 and Figure 4]. These results are useful for investigating specific processes, functions, and pathways involved in sanguinarine-treated ovarian cancer.

DISCUSSION

Ovarian cancer is a common gynecological malignancy that threatens female health seriously, owing to the high recurrence and the poor clinical outcomes.[1-3] It has been stated that sanguinarine has antioxidant, anti-inflammatory, pro-apoptotic, and growth inhibitory effects on a variety of cancer cells.[7] However, the underlying mechanisms of sanguinarine in ovarian cancer remain indefinable. In the present study, we discovered the mechanism underlying the antitumor activity of sanguinarine in ovarian cancer by gene microarray assay and integrated bioinformatics analysis. As a total, 1185 DEGs, 835 were upregulated and 350 were downregulated. Then, we performed GO annotation and KEGG pathway enrichment analysis of the 1185 DEGs. Among GO annotation enrichment analysis of the sanguinarine target genes, BP term annotation is the most augmented term. In addition, cytoskeleton, ER, genetic material, and nuclear and extracellular space were suggestively identified in CC term annotation. In MF term annotation, the target genes were significantly attentive on the activity of transcriptional activators, protein dimerization/heterodimerization activity, as well as the binding of enzyme, DNA, ribonucleotide, identical protein, and regulatory region nucleic acid.

Top 10 upregulated genes, including HSPA7, HSPA6, ATF3, CRYAB, LAMA2, ANKRD1, HMOX1, C20orf197, GEM, and KLF4, were recognized based on the microarray of DEG analyses. HSPA7 and HSPA6 are members of the human heat shock protein gene family, which are significant regulators of cellular proliferation, differentiation, and strongly occupied in the molecular orchestration of cancer development and progression.[17,18] HSPA6 is one of the five major HSP70 members. HSP70 proteins are auspicious drug targets for cancer therapy and they are involved in mediating drug resistance in cancer therapy. The expression of HSPA6 can be induced by anticancer agents.[19,20] Emily *et al.* stated that proteasome inhibitor MG-132 induces the expression of HSPA6 on the surface of human colon cancer cells.[21] Petric *et al.* found that potential anticancer agents such as HSP90 inhibitors, geldanamycin, induce the expression of HSPA6 in breast cancer cells, signifying that HSPA6 may be a specific marker for HSP90 inhibition.[22] In the present study, the expression

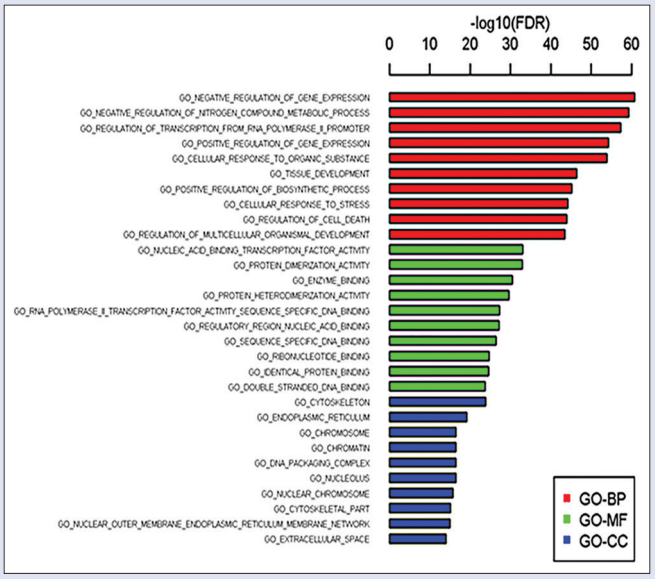

Figure 3: Gene annotation (gene ontology) enrichment analysis of differentially expressed genes (top 10). The bar plot shows the enrichment scores (−log10[false discovery rate]) of significant enrichment gene ontology terms. Gene ontology categories cover three domains: biological processes, molecular functions and cellular components (false discovery rate <0.05 is recommended)

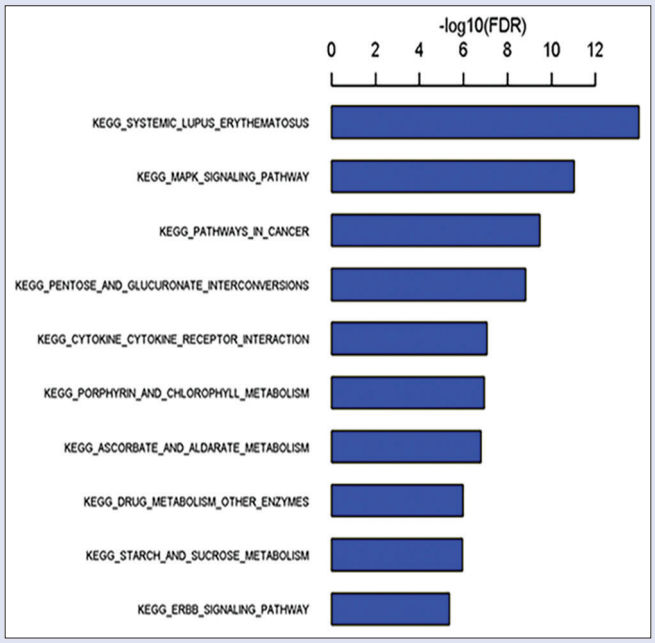

Figure 4: Kyoto Encyclopedia of Genes and Genomes signal pathway analysis of differentially expressed genes (top 10). The bar plot shows the enrichment scores (−log10[false discovery rate]) of significant enrichment Kyoto Encyclopedia of Genes and Genomes pathway analysis (false discovery rate <0.05 is recommended)

of HSPA6 was elevated, which indicated that it may also serve as a specific marker of sanguinarine-treated ovarian cancer compared with control groups. The relevant underlying mechanism, however, requires further investigation. The other genes such as DHRS3, SDPR, GNB4, MARCKS, and MMP7 were downregulated. DHRS3 is a member of the SDR family and was identified as a novel transcriptional target of the p53 family.[23] To the best of our knowledge, the study of DHRS3 in cancer is inadequate. A high expression level of DHRS3 has been detected in a variety of cancers, including hepatocellular carcinomas,[24] papillary thyroid carcinomas,[25,26] and neuroblastomas.[27] These data recommended that DHRS3 may serve as an oncogene to promote the occurrence of cancer. The second downregulated gene identified in the present study was SDPR, which is a member of the Cavin protein family. SDPR has not been reported in ovarian cancer but has been stated in other types of tumors. A previous study stated that SDPR functions as a tumor suppressor gene in breast, thyroid, liver, kidney, and prostate cancer.[28-31] Moreover, Ozturk *et al*. described that SDPR functions as a metastasis suppressor in breast cancer by promoting apoptosis of breast cancer cells.[32] However, Tahara *et al*. found that SDPR is related to tumor progression in endometrioid cancer.[33] Scholars speculate that SDPR-mediated signal activation varies according to the type of cancer. Up to now, it has not been reported the relationships between SDPR and ovarian cancer.

In addition, the KEGG analysis results recommended that the DEGs were identified in "Systemic lupus erythematosus," "MAPK signalling pathway," "Pathways in cancer," and "metabolic pathways" [Table S5]. Recent studies conveyed that systemic lupus erythematosus might be an independent risk factor for cancer.[34] MAPK signaling pathway was linked with differentiation, proliferation, and apoptosis of cells and its family members mainly include extracellular signal-regulated kinase (ERK), stress-activated protein kinase (JNK), and p38 mitogen-activated protein kinase (p38MAPK).[35] Lee *et al*. reported that MAPK/ERK signaling pathway might play a role in the development and progression of ovarian cancer and might provide a novel option for molecular-targeted cancer therapies.[36] Furthermore, another study disguised that activating "MAPK signalling pathway" served an important role in ovarian cancer through inducing cell apoptosis and protective autophagy.[37] Previous studies established that "Pathways in cancer" and "Metabolic pathways" were linked with tumor incidence and tumorigenesis.[38-40]

CONCLUSION

The present study has identified DEG, GO annotation, and KEGG pathway enrichment analysis between sanguinarine and control groups that may help us to disclose the mechanism underlying sanguinarine target genes in ovarian cancer cells, evaluate the potential therapeutic target of OC patients, and provide more clues for ovarian cancer treatment. Further studies are required to clarify molecular pathogenesis and alteration in signaling pathways of these genes involved sanguinarine-treated ovarian cancer. Sanguinarine may serve as an effective therapeutic reagent for epithelial ovarian cancer.

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Conflicts of interest

There are no conflicts of interest.

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Table S1: Upregulated and downregulated genes in differential expression (top 10)

Index	Entrez	Gene symbol	Fold change	Regulation	*P*	FDR
			Upregulated genes			
1	3311	HSPA7	396.6446648	Up	2.394E-10	3.968E-07
2	3310	HSPA6	396.6446648	Up	2.394E-10	3.968E-07
3	467	ATF3	141.7095654	Up	1.7465E-11	2.428E-07
4	1410	CRYAB	115.9203463	Up	1.0681E-10	3.383E-07
5	3908	LAMA2	110.6523285	Up	3.8307E-11	2.987E-07
6	27,063	ANKRD1	104.2974446	Up	2.8037E-11	2.733E-07
7	3162	HMOX1	97.49389662	Up	4.9516E-11	3.217E-07
8	284,756	C20orf197	85.50498732	Up	4.3963E-08	3.094E-06
9	2669	GEM	83.31282348	Up	6.349E-10	4.868E-07
10	9314	KLF4	58.26307739	Up	1.089E-10	3.383E-07
			Downregulated genes			
1	9249	DHRS3	−9.41384	Down	9.9807E-07	1.771E-05
2	8436	SDPR	−8.45886052	Down	4.9995E-09	1.091E-06
3	59,345	GNB4	−7.67918569	Down	2.035E-06	2.686E-05
4	4082	MARCKS	−7.40476183	Down	4.074E-08	2.963E-06
5	4316	MMP7	−7.39788736	Down	4.5177E-09	1.006E-06
6	3852	KRT5	−7.31835804	Down	6.9333E-09	1.209E-06
7	51,313	FAM198B	−7.10069382	Down	3.8329E-08	2.847E-06
8	84,281	C2orf88	−6.91875921	Down	5.4551E-09	1.115E-06
9	51,473	DCDC2	−6.40015421	Down	6.9825E-09	1.209E-06
10	23,112	TNRC6B	−6.27831174	Down	5.207E-07	1.214E-05

FDR: False discovery rate

Table S2: Gene ontology (biological process) enrichment analysis of differentially expressed genes (top 10)

Index	Gene set name	Genes in overlap	*P*	FDR
1	GO: Negative regulation of gene expression	167	3.63E-65	1.69E-61
2	GO: Negative regulation of nitrogen compound metabolic process	166	2.23E-63	5.2E-60
3	GO: Negative regulation of transcription from RNA polymerase IIpromoter	177	2.95E-61	4.58E-58
4	GO: Positive regulation of gene expression	170	4.98E-58	5.79E-55
5	GO: Cellular response to organic substance	175	1.49E-57	1.39E-54
6	GO: Tissue development	148	5.66E-50	4.39E-47
7	GO: Positive regulation of biosynthetic process	160	1.15E-48	7.63E-46
8	GO: Cellular response to stress	147	1.21E-47	7.02E-45
9	GO: Regulation of cell death	142	2.34E-47	1.21E-44
10	GO: Regulation of multicellular organismal development	151	7.5E-47	3.49E-44

GO: Gene ontology; FDR: False discovery rate

Table S3: Gene ontology (cellular component) enrichment analysis of differentially expressed genes (top 10)

Index	Gene set name	Genes in overlap	*P*	FDR
1	GO: Cytoskeleton	131	2.89E-27	1.69E-24
2	GO: Endoplasmic reticulum	108	2.39E-22	6.98E-20
3	GO: Chromosome	71	1.66E-19	3.09E-17
4	GO: Chromatin	49	2.11E-19	3.09E-17
5	GO: DNA packaging complex	26	2.85E-19	3.33E-17
6	GO: Nucleolus	69	3.49E-19	3.4E-17
7	GO: Nuclear chromosome	52	2.18E-18	1.82E-16
8	GO: Cytoskeletal part	91	9.68E-18	7.06E-16
9	GO: Nuclear outer membrane endoplasmic reticulum membrane network	73	1.62E-17	1.05E-15
10	GO: Extracellular space	86	1.82E-16	1.06E-14

GO: Gene ontology; FDR: False discovery rate

Table S4: Gene ontology (molecular function) enrichment analysis of differentially expressed genes (top 10)

Index	Gene set name	Genes in overlap	*P*	FDR
1	GO: Nucleic acid-binding transcripition factor activity	113	1.13E-36	1.05E-33
2	GO: Protein dimerization activity	110	2.54E-36	1.18E-33
3	GO: Enzyme binding	133	1.21E-33	3.75E-31
4	GO: Protein heterodimerization activity	67	1.14E-32	2.64E-30
5	GO: RNA polymerase II transcription factor activity sequence-specific DNA binding	74	2.71E-30	5.04E-28
6	GO: Regulatory region nucleic acid binding	84	5.32E-30	8.24E-28
7	GO: Sequence specific DNA binding	94	2.81E-29	3.73E-27
8	GO: Ribonucleotide binding	127	2.01E-27	2.33E-25
9	GO: Identical protein binding	99	2.48E-27	2.56E-25
10	GO: Double-stranded DNA binding	76	2.39E-26	2.22E-24

GO: Gene ontology; FDR: False discovery rate

Table S5: Kyoto Encyclopedia of Genes and Genomes signal pathway analysis of differentially expressed genes (top 10)

Index	Pathway	Genes in overlap	*P*	FDR
1	Systemic lupus erythematosus	27	2.61E-17	1.05E-14
2	MAPK signaling pathway	32	4.59E-14	9.25E-12
3	Pathways in cancer	33	2.59E-12	3.48E-10
4	Pentose and glucuronate interconversions	11	1.53E-11	1.54E-09
5	Cytokine–cytokine receptor interaction	26	1.07E-09	8.63E-08
6	Porphyrin and chlorophyll metabolism	11	1.71E-09	0.000000115
7	Ascorbate and aldarate metabolism	9	2.82E-09	0.000000162
8	Drug metabolism - other enzymes	11	2.09E-08	0.00000105
9	Starch and sucrose metabolism	11	2.59E-08	0.00000116
10	ErbB signaling pathway	13	0.000000113	0.00000457

FDR: False discovery rate; MAPK: Mitogen-activated protein kinase